With extracts containing radioactive amino acids and prepared from the livers of mice injected with [U-^{14}C]-glucose the procedure was simplified. The impure mixture of TAB amino acids was applied to the silica gel column, which was washed with 100 ml of light petroleum (b.p. 60–80°), and then the TAB derivatives were eluted with 50 ml of diethyl ether. Also, standard TAB amino acids may become contaminated during preparation and storage but may be readily purified before use by silica gel chromatography.

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CHROM. 3927

Amino acid micro determination with a standard single-column analyzer

In investigations of protein structure the amount of material available for quantitative amino acid analysis and degradation as well as the rate of analysis per day if often a limiting factor. Therefore, it was advantageous to have a single-column system available for the determination of all protein constituent amino acids from one sample, as was described first by PIEZ AND MORRIS[1]. Instruments based on this principle are commercially available (Phoenix Precision Instrument Co., Philadelphia, Pa; Technicon Instrument Co., Ltd., Hanworth Lane, Chertsey, Surrey). Previously, for a complete analysis at least 0.1 μmole of sample had to be applied to the top of a column 0.6 cm (or more) in internal diameter, though only part (30% or less) of the resolved material was used for quantitative detection by the analytical system. BYFIELD[2] described the use of a column 0.3 × 120 cm for the usual 22-hour chromatogram, which permitted sub-micro determination in connection with a voltage amplifier attached to the flow cell colorimeter.

In this paper a similar set of modifications for a standard model of an amino

acid analyzer will be described that enables the apparatus to complete a chromatogram on quantities as low as 0.01 μmole within 11 h without electrical amplification. A column system operating with time switches readily enables two analyses to be carried out daily with no loss of resolution for the protein amino acids. For still greater sensitivity a voltage amplifier may be attached to the colorimeters according to BYFIELD[2].

Methods and materials

The instrument is a standard apparatus (Technicon Auto Analyzer) except that it is equipped with a micro column, 0.3 × 120 cm. The resin is Chromobead ® type C-2, 13 ± 1 μ sperical particles. The developing buffer is pumped by a Milton Roy pump at a volumetric input of 0.21 ml/min, which develops about 350–400 p.s.i. pressure on the column during a run. The column effluent line feeds directly into the nitrogen-segmented ninhydrin stream after the proportioning pump by means of a T piece (Technicon H-3 piece). Therefore all of the resolved material is used for detection. The proportioning pump lines for the nitrogen and ninhydrin stream are approximately one quarter of the usual size, since the column cross-sectional area is a quarter of the usual. The pumping tubes for the proportioning pump are commercially available and deliver 0.16 ml/min of nitrogen and 0.42 ml/min of ninhydrin, while 0.42 ml/min are sucked through the flow cells of the colorimeters by means of a third pump line. Since smaller volumes are delivered into the analytical system, the heating coil for ninhydrin color development had to be shortened to 550–600 cm, giving a heating time of approx. 17 min. All other parts, such as flow cell colorimeters and electrical recording system, remain unchanged. The light path of the longitudinal type flow cells remained 10 mm as usual; however, in the chromatogram shown, the 440-mμ cell had a 5-mm light path. Therefore the proline content of the standard mixture was increased to 0.025 μmole for this chromatogram. The use of a 10-mm light path (or more) is recommended. From the Autograd a modified gradient is supplied with the composition given in Table I.

Results and discussion

The resolution of a typical chromatogram of a standard mixture of 0.02 μmole

TABLE I

GRADIENT (IN ml) FOR 10.5-HOUR CHROMATOGRAM

Chamber	0.05 *M* sodium citrate ([Na^+] = 0.2 *M*)			[Na^+] = 1.2 *M*	[Na^+] = 1.8 *M*
	pH 2.75	*pH 2.875*	*pH 3.80*	*pH 10.00*	*pH 11.50*
1	15.5 2.0 MeOH				
2	13.0	4.5			
3		12.5	5.0		
4			17.5		
5			17.5		
6			11.5	6.0	
7				15.5	2.0
8					17.5
9					17.5

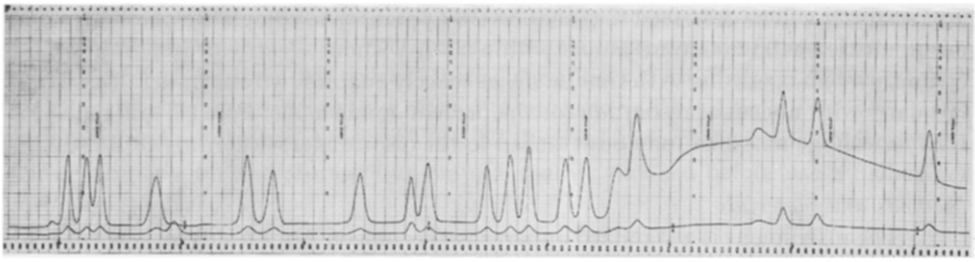

Fig. 1. Typical chromatogram of a standard mixture of 0.02 μmole each of the protein constituent amino acids. Region Asp to Arg. No range expansion. (Pro 0.025 μmole.)

each of the protein constituent amino acids is shown in Fig. 1. A peak representing height is obtained for each amino acid as in the usual chromatogram. The norleucine equivalents differ from the usual quoted values because of the prolonged ninhydrin color development in the heating bath. Repeated blocking of the Teflon sinter at the column outlet due to the narrow particle size range of the resin, could be cured by topping the sinter with a small layer of 1–2 mm of type A resin before packing the column with the Chromobead type C-2 resin. This did not affect the resolution. The column is regenerated by washing with 0.5 *N* sodium hydroxide for at least 1 h, and thereafter it is reequilibrated with a pH 2.875 buffer for as long as is necessary to bring the eluate to the same pH. When not in use, the column is kept under 0.5 *N* sodium hydroxide. This procedure should be followed in order to keep the ammonia baseline down and out of the phenylalanine peak. After the column has been loaded with the sample for analysis, it is filled to the top with the pH 2.875 buffer containing 6% of methanol. The pump lines from the Autograd to the column top are filled with the same buffer (6% in methanol) before they are connected to the Autograd, and the analysis is started. A column prepared in this manner has been in use in our laboratory for more than half a year without repacking. During this time the resin settled about 5 cm, resulting in a slight increase in operating pressure of about 50–100 p.s.i. and extending the time necessary for chromatography by 30–60 min up to 10.5 h. Because the resolution is very sensitive to small changes in buffer pH, buffers have to be carefully stored in a cool place and checked and readjusted if necessary. The reproducibility is ± 5% for duplicates.

It may be concluded that with very little additional equipment an existing apparatus can be altered to gain in sensitivity and to double the number of micro analyses to be carried out daily.

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CHROM. 3924

Desalting of nucleic acid hydrolysates, nucleosides and bases by chromatography on poly-N-vinyl pyrrolidone*

It is often desirable to separate nucleic acid components from salts used in their isolation. Methods available include use of charcoal[1–3], anion exchangers[4,5], cross-linked dextrans[6], polyacrylamide gels[7–9], and extraction with an acetone–ethanol mixture[10,11]. All of these have certain merit, but also possess a number of disadvantages.

We have recently reported on the use of insoluble poly-N-vinyl pyrrolidone for the fractionation of certain nucleotide derivatives[12]. This technique has now been extended and found to be a practical substitute for the above mentioned methods of desalting nucleotides, nucleosides, purines, and pyrimidines. Distilled water is used as eluant, compounds are quantitatively recovered in small volumes, and the bases show clean spectra. No regeneration of the column is required, and it may be used repeatedly for a number of experiments.

Materials and methods

RNA (Na salt, purified from Torula) was obtained from Calbiochem**. DNA (sperm), bases, nucleosides, and nucleotides were purchased from Nutritional Biochemical Co., and were used without further purification. Insoluble PVP (GAF Corporation, New York), sold under the trade name of Polyclar AT Powder, was used in these studies.

The Polyclar AT was mixed with distilled water, and the fines were discarded by repeated decantation. The suspension was poured into a column and allowed to pack with gravity flow. The bed was supported by glass wool.

Hydrolysis of DNA was carried out by heating 20 mg DNA in 1 ml 0.1 *M* H_2SO_4 at 100° for 35 min in a sealed tube. After neutralization with NH_4OH and centrifugation, a 0.5 ml aliquot was added to 0.5 ml 10 *N* LiCl. A 0.3 ml aliquot of this (equivalent to 2.5 mg DNA) was then applied to the PVP column.

RNA was hydrolyzed by heating 4 mg RNA in 0.5 ml 1 *N* HCl at 100° for 1 h. The pH was adjusted to 7 with 1 *N* NaOH and 0.2 ml was applied to the column.

Tests of the separation of bases and nucleosides from ammonium sulfate were

* The following abbreviations will be used: CMP = cytidine monophosphate; UDP = uridine diphosphate; RNA = ribonucleic acid; DNA = deoxyribonucleic acid; PVP = poly-N-vinyl pyrrolidone.

** Mention of trade or company names does not imply endorsement by the Department over others not named.